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Grafting of vinyl monomers to collagen by ceric ion

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Collagen, a versatile fibre, still maintains its primary position in the production of leather. Recent years have, however, seen increasing inroads into the leather markets by substitute materials from the plastic and synthetic polymer industry.

Grafting of vinyl monomers to natural and synthetic polymers by means of chemical or radiation initiated polymerisation has been suggested as a potentially good means of altering the properties of the base polymer.[1] Graft polymerisation of vinyl type monomers onto cotton, starch, cellulose wool, etc., has been investigated rather extensively by means of chemical or radiation initiated polymerisation during the past several years.[2-6] It is surprising, therefore, that comparatively little attention has been paid to chemical or radiation initiated grafting to collagen fibres. Hence as part of a research programme designed to expand the industrial markets for hide protein collagen and collagen based products such as leather, investigations were taken up to modify collagen by graft copolymerisation with various vinyl monomers.

In the graft polymerisation process many initiation techniques such as chemical and high energy radiation can be employed. In a preliminary evaluation of initiating systems for grafting vinyl monomers to collagen, ceric ion was first chosen for a more intensive study. The Redox method with ceric salts, particularly ceric ion alcohol systems, has been used for initiation of polymerisation and grafting of vinyl monomers, and the kinetics and mechanism of the reaction have been studied in detail by a number of investigators.[7,8,10,11] Results from model experiments designed to elucidate the mechanism of grafting to cellulose by ceric salts indicate that grafting is initiated by free radicals either at the α-carbon atom of the primary alcohol or at the α-carbon atom of the 1,2-glycol in the glucose unit;[9] the same mechanism was also therefore expected to apply to the collagen-ceric ion system. Collagen contains alcoholic groups at serine, threonine, hydroxyproline and hydroxylysine residues. It has been shown that ceric ions complex reversibly with alcohols and glycols and that the dissociation of the complexes is the rate determining step.[7] In the case of alcohols, the mechanism of the initiation reaction can be given generally as follows:

$$Ce^{IV} + R\ CH_2OH \underset{}{\overset{K}{\rightleftarrows}} B \xrightarrow{Kd}$$

$$Ce^{III} + H^+ + R\ \dot{C}HOH \text{ or } (R\ CH_2\dot{O})$$

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where Ce^{IV} represents the ceric complexes as they exist in aqueous solution. B the ceric alcohol complex, and R $\dot{C}$HOH a free radical.

The most important feature of the oxidation with ceric ion is that it proceeds via a single electron transfer with the formation of free radicals on the reducing agent.[7,10,11] Thus, if the reducing agent is a polymeric molecule such as cellulose, or collagen, and the oxidation is carried out in the presence of a vinyl monomer the free radical produced on the polymeric molecule (backbone) initiates polymerisation to produce a graft copolymer.

Experimental and results

One of the factors which may influence the grafting of monomers to collagen will be the rate of diffusion of the monomers into collagen. Since swollen and soluble collagen will be more accessible to both monomer and catalyst grafting experiments were first carried out after depolymerising the insoluble collagen by the Nishihara technique using α-amylase as reported by Steven.[12] The technique consists of treating the insoluble collagen by α-amylase and then dispersing the treated collagen in a large volume of 0·2M acetic acid. For grafting on insoluble collagen, hide powder was used. It was soaked in water or the appropriate solvent for a few hours before subjecting to grafting. All the grafting experiments were carried out in nitrogen atmosphere at the laboratory temperature. Various experimental factors like monomer concentration, initiator concentration, manner of addition of initiator and monomer, time and temperature, influence the process of copolymerisation. In the present work, acrylonitrile and methylmethacrylate were used for the grafting experiments. 100 ml. of solvent (water-N, N-dimethyl formamide, 1:1 v/v in the case of acrylonitrile and water in the case of methylmethacrylate) was used per 0·05 unit mole residue weight of collagen. The monomer concentration used was 0·1 mole and 5 ml. of 0·1M ceric ammonium nitrate (A.R.) in 1N nitric acid was used as initiator. The reaction was allowed to proceed for a known time.

After grafting, the monomer was removed by washing with distilled water and the loosely bound polymer was removed by extraction of product for 72 hours at room temperature with dimethyl formamide in the case of acrylonitrile grafted collagen, and dichloroethane in the case of methyl methacrylate grafted collagen.

A typical graft copolymer of polymethyl methacrylate on collagen was prepared as described above using insoluble hide collagen and the product was analysed for total nitrogen, arginine and hydroxyproline. Total nitrogen was determined by the Kjeldahl method, arginine by the modified method of Macpherson[13] and hydroxyproline by the method of Newman and Logan.[14] The collagen content in the graft copolymer was then calculated from these data and the results obtained are presented in Table 1. A number of other graft copolymers were also prepared using solubilised collagen and other vinyl monomers and the results of analyses of these samples will be reported later.

Discussion

From Table 1, it can be seen that the collagen content obtained by the arginine and total nitrogen method agree within reasonable limits. The values obtained by these two methods indicate the percent grafting to be about 103. The increase in weight by the original weight X 100 was recorded as the percent grafting. As a further proof of grafting, the grafted collagen was hydrolysed with 6N HCl over a water bath and the graft was isolated and tested with ninhydrin reagent which gave the characteristic blue colour normally associated with the presence of amino acids.

Table 1

ESTIMATION OF TOTAL NITROGEN AND CERTAIN AMINO ACIDS IN THE COLLAGEN-METHYL METHACRYLATE GRAFT

	g./100 g.	% collagen in the graft
Total nitrogen	8·8	49·6
Arginine	4·1	48·0
Hydroxyproline	5·0	37·0

The above experiments conclude that actual grafting takes place and the acid hydrolysis leaves behind the graft containing attached amino acid residues. In the grafting of vinyl monomers to collagen, the graft is attached to certain amino acids through -C-C- linkage. When the product is therefore hydrolysed with hydrochloric acid, the amino acid through which the grafting has taken place will still be attached to the graft and this will be removed from the hydrolysates. The lower values obtained for hydroxyproline therefore indicate that some of the grafts are attached to hydroxyproline residues. The effect of swelling agents on the rate of grafting to swollen fibre structures has been widely interpreted as due to the faster rate of diffusion of the monomer to the active centres formed in the swollen fibre.[4] Hence in the present study, the solvent systems were chosen in such a way they act both as a solvent for the homopolymer and as a swelling agent. Once the proof of grafting was established, the graft can be isolated and characterised with respect to the degree of polymerisation and a number of other properties examined; these studies are in progress.

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